FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE, CANCERLIT' ENTERED AT 09:28:50 ON 24 JUL 2003

L1	9	REGULATABLE RIBOZYME
L2	132	ALLOSTERIC RIBOZYME
L3	3111	GROUP I INTRON
L4	8278	REGULATORY DOMAIN
L5	1	ALLOSTERIC POLYNUCLEOTIDE
L6	1	L1 AND L3
L7	0	L2 AND L3 AND L4
L8	3	L2 AND L3
L9	3	DUP REM L8 (0 DUPLICATES REMOVED)
L10	132	ALLOSTER? RIBOZYM?
L11	1	ALLOSTER? POLYNUCLEOTIDE
L12	17060	RIBOZYM?
L13	1051	L12 AND L3
L14	0	L13 AND L4
L15	47	APTAZYM?
L16	3	L15 AND L3
L17	3	DUP REM L16 (0 DUPLICATES REMOVED)
L18	21	THYMIDYLATE SYNTHASE INTRON
L19	238	TD INTRON
L20	93437	THEOPHYLLIN?
L21	0	L18 AND L20
L22	2	L19 AND L20
L23	631	SELF SPLICING INTRON?
L24	2	L23 AND L20
L25	2	L23 AND SMALL ORGANIC EFFECTO?
L26	1	DUP REM L25 (1 DUPLICATE REMOVED)

L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:261999 CAPLUS
DOCUMENT NUMBER: 138:282303
TITLE: **Regulatable ribozymes** and DNAzymes and their use in regulation of cellular product levels or screening for cells producing particular bioproducts
INVENTOR(S): Wilson, Charles; Cload, Sharon T.; Keefe, Anthony D.
PATENT ASSIGNEE(S): Archemix Corporation, USA
SOURCE: PCT Int. Appl., 128 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003027310	A2	20030403	WO 2002-US30458	20020924
WO 2003027310	A3	20030626		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-324715P P 20010924

AB Compns. and methods are provided to make, isolate, characterize and use regulatable, catalytically active nucleic acids (RCANA). The present invention is directed to RCANA that transduce mol. recognition into catalysis. Also, RCANAs according to the invention can be used as regulatory elements to control the expression of one or more genes in a metabolic pathway. RCANAs can also be used as regulated selectable markers to create a selective pressure favoring (or disfavoring) prodn. of a targeted bioproduct. In addn., the RCANAs can be used to regulate the activity of a reporter gene in cells and thereby provide a means to screen a population of cells for a cell producing a desired bioproduct. Thus, a selection scheme to provide protein-**regulatable ribozymes** was developed and applied to tyrosyl-tRNA synthetase-regulated **group I intron** ND1 of Neurospora to produce hen egg white lysozyme-regulated ligase. This ribozyme exhibited a 3100-fold activation by lysozyme, ligating with a rate of 0.6 h-1 in the presence of lysozyme but only 0.0002 h-1 in its absence.

L9 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:232387 CAPLUS
DOCUMENT NUMBER: 138:265004
TITLE: RNA in drug development
AUTHOR(S): Kozu, Tomoko
CORPORATE SOURCE: Saitama Cancer Cent. Res. Inst., Japan
SOURCE: Tanpakushitsu Kakusan Koso (2003), 48(4, 3Gatsugozoka), 540-548
CODEN: TAKKAJ; ISSN: 0039-9450
PUBLISHER: Kyoritsu Shuppan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review on the principle and clin. application of RNA-based drugs and biosensors, discussing: (1) gene knockdown by using antisense oligonucleotides, ribozymes, dsRNA, siRNA, and group II intron, (2) RNA repair by trans-splicing using **group I intron** and spliceosome, (3) functional modification of proteins (VEGF, coagulation factor IXa, etc.) by RNA aptamers, and (4) RNA-based biosensors using **allosteric ribozymes**, aptazymes, and aptamers.

L9 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:22547 CAPLUS
DOCUMENT NUMBER: 138:282224
TITLE: Group I aptazymes as genetic regulatory switches
AUTHOR(S): Thompson, Kristin M.; Syrett, Heather A.; Knudsen, Scott M.; Ellington, Andrew D.
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, 78712, USA
SOURCE: BMC Biotechnology [online computer file] (2002), 2, No pp. given
CODEN: BBMIE6; ISSN: 1472-6750
URL: http://www.biomedcentral.com/1472-6750/2/21
PUBLISHER: BioMed Central Ltd.
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English
AB **Allosteric ribozymes** (aptazymes) that have extraordinary activation parameters have been generated in vitro by design and selection. For example, hammerhead and ligase ribozymes that are activated by small org. effectors and protein effectors have been selected from random sequence pools appended to extant ribozymes. Many ribozymes, esp. self-splicing introns, are known control gene regulation or viral replication in vivo. We attempted to generate Group I self-splicing introns that were activated by a small org. effector, theophylline, and to show that such Group I aptazymes could mediate theophylline-dependent splicing in vivo. By appending aptamers to the Group I self-splicing intron, we have generated a Group I aptazyme whose in vivo splicing is controlled by exogenously added small mols. Substantial differences in gene regulation could be obsd. with compds. that differed by as little as a single Me group. The effector-specificity of the Group I aptazyme could be rationally engineered for new effector mols. In conclusion, group I aptazymes may find applications as genetic regulatory switches for generating conditional knockouts at the level of mRNA or for developing economically viable gene therapies.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:924000 CAPLUS
DOCUMENT NUMBER: 136:66194
TITLE: Methods for selection and use of regulatable, catalytically active nucleic acids (RCANA) or aptazymes

INVENTOR(S): Ellington, Andrew D.; Hesselberth, Jay; Marshall, Kris; Robertson, Michael; Sooter, Letha; Davidson, Eric; Cox, J. Colin; Reidel, Timothy

PATENT ASSIGNEE(S): Board of Regents the University of Texas System, USA

SOURCE: PCT Int. Appl., 126 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096559	A2	20011220	WO 2001-US19302	20010614
WO 2001096559	A3	20030710		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-212097P P 20000615

AB Compns. and methods are provided to make, isolate, characterize and use regulatable, catalytically active nucleic acids (RCANA). RCANA may be used for regulating gene expression and in assays to detect the presence of ligands or to detect activation by an effector of an RCANA bound to a solid support such as a chip or multi-well plate. Also disclosed are compns. and methods for automating the selection procedures of the present invention. In addn., the invention claims diagnostic and therapeutic applications. One example of the invention involves construction of an RCANA by PCR using primers from the P6 region of the Group I ribozyme, cloning of the RCANA or in vitro transcription followed by RNA purifn., and demonstration of theophylline-dependent splicing activity towards the bacteriophage T4 gene td intron in vivo or in vitro. As another example of the invention, an RCANA was isolated with an activity that was increased 75,000-fold in the presence of its protein effector, Neurospora crassa mitochondrial tyrosyl tRNA synthetase (Cyt18). This RCANA was selected from a pool of randomized sequences spanning the catalytic core of L1 ligase by selecting for the ability to ligate an oligonucleotide tag in the presence of the Cyt18 effector and affinity capture of the oligonucleotide tag. The in vitro selection can be automated by immobilization of targets on beads and high-stringency washes to remove non-binding species. Activity of another protein-dependent ribozyme was increased 3,500-fold in the presence of hen egg white lysozyme. The lysozyme-dependent ribozyme was also activated by turkey egg white lysozyme but not by T4 lysozyme and was inhibited by a lysozyme-specific RNA binding species. A peptide-dependent RCANA was isolated with an 18,000-fold increase in its activity in the presence of the arginine-rich motif (ARM) from the HIV-1 Rev protein but not the ARM from HTLV-I Rex protein.

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:424260 CAPLUS
DOCUMENT NUMBER: 129:78818
TITLE: Biosensors based on bioreactive **allosteric polynucleotides**, RNA ribozymes and catalytic DNA
INVENTOR(S): Breaker, Ronald R.
PATENT ASSIGNEE(S): Yale University, USA; Breaker, Ronald R.
SOURCE: PCT Int. Appl., 107 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9827104	A1	19980625	WO 1997-US24158	19971218
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9858107	A1	19980715	AU 1998-58107	19971218
AU 724627	B2	20000928		
EP 958303	A1	19991124	EP 1997-954296	19971218
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002514913	T2	20020521	JP 1998-528049	19971218

PRIORITY APPLN. INFO.:
US 1996-33684P P 19961219
US 1997-55039P P 19970808
WO 1997-US24158 W 19971218

AB This invention relates primarily to functional DNA polynucleotides that exhibit allosteric properties and to catalytic RNA and DNA polynucleotides that have catalytic properties with rates that can be controlled by a chem. effector, a phys. signal, or combinations thereof. In some preferred embodiments, the polynucleotides are DNA enzymes that are used in soln. or in suspension or are attached to a solid support as biosensors to detect the presence or absence of a compd., its concn., or phys. change in a sample by observation of self-catalysis. Chem. effectors include org. compds. such as amino acids, amino acid derivs., peptides, nucleosides, nucleotides, steroids, and mixts. of these with each other and with metal ions, cellular metabolites or blood components obtained from biol. samples, steroids, pharmaceuticals, pesticides, herbicides, food toxins, and the like. Phys. signals include radiation, temp. changes, and combinations thereof.

L17 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:232387 CAPLUS
DOCUMENT NUMBER: 138:265004
TITLE: RNA in drug development
AUTHOR(S): Kozu, Tomoko
CORPORATE SOURCE: Saitama Cancer Cent. Res. Inst., Japan
SOURCE: Tanpakushitsu Kakusan Koso (2003), 48(4, 3Gatsugozoka), 540-548
CODEN: TAKKAJ; ISSN: 0039-9450
PUBLISHER: Kyoritsu Shuppan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review on the principle and clin. application of RNA-based drugs and biosensors, discussing: (1) gene knockdown by using antisense oligonucleotides, ribozymes, dsRNA, siRNA, and group II intron, (2) RNA repair by trans-splicing using **group I intron** and spliceosome, (3) functional modification of proteins (VEGF, coagulation factor IXa, etc.) by RNA aptamers, and (4) RNA-based biosensors using allosteric ribozymes, **aptazymes**, and aptamers.

L17 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:22547 CAPLUS
DOCUMENT NUMBER: 138:282224
TITLE: Group I **aptazymes** as genetic regulatory switches
AUTHOR(S): Thompson, Kristin M.; Syrett, Heather A.; Knudsen, Scott M.; Ellington, Andrew D.
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, 78712, USA
SOURCE: BMC Biotechnology [online computer file] (2002), 2, No pp. given
CODEN: BBMIE6; ISSN: 1472-6750
URL: http://www.biomedcentral.com/1472-6750/2/21
PUBLISHER: BioMed Central Ltd.
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English
AB Allosteric ribozymes (**aptazymes**) that have extraordinary activation parameters have been generated in vitro by design and selection. For example, hammerhead and ligase ribozymes that are activated by small org. effectors and protein effectors have been selected from random sequence pools appended to extant ribozymes. Many ribozymes, esp. self-splicing introns, are known control gene regulation or viral replication in vivo. We attempted to generate Group I self-splicing introns that were activated by a small org. effector, theophylline, and to show that such Group I **aptazymes** could mediate theophylline-dependent splicing in vivo. By appending aptamers to the Group I self-splicing intron, we have generated a Group I **aptazyme** whose in vivo splicing is controlled by exogenously added small mols. Substantial differences in gene regulation could be obsd. with compds. that differed by as little as a single Me group. The effector-specificity of the Group I **aptazyme** could be rationally engineered for new effector mols. In conclusion, group I **aptazymes** may find applications as genetic regulatory switches for generating conditional knockouts at the level of mRNA or for developing economically viable gene therapies.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:924000 CAPLUS
DOCUMENT NUMBER: 136:66194
TITLE: Methods for selection and use of regulatable, catalytically active nucleic acids (RCANA) or

aptazymes

INVENTOR(S): Ellington, Andrew D.; Hesselberth, Jay; Marshall, Kris; Robertson, Michael; Sooter, Letha; Davidson, Eric; Cox, J. Colin; Reidel, Timothy

PATENT ASSIGNEE(S): Board of Regents the University of Texas System, USA

SOURCE: PCT Int. Appl., 126 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096559	A2	20011220	WO 2001-US19302	20010614
WO 2001096559	A3	20030710		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-212097P P 20000615

AB Compns. and methods are provided to make, isolate, characterize and use regulatable, catalytically active nucleic acids (RCANA). RCANA may be used for regulating gene expression and in assays to detect the presence of ligands or to detect activation by an effector of an RCANA bound to a solid support such as a chip or multi-well plate. Also disclosed are compns. and methods for automating the selection procedures of the present invention. In addn., the invention claims diagnostic and therapeutic applications. One example of the invention involves construction of an RCANA by PCR using primers from the P6 region of the Group I ribozyme, cloning of the RCANA or in vitro transcription followed by RNA purifn., and demonstration of theophylline-dependent splicing activity towards the bacteriophage T4 gene td intron in vivo or in vitro. As another example of the invention, an RCANA was isolated with an activity that was increased 75,000-fold in the presence of its protein effector, Neurospora crassa mitochondrial tyrosyl tRNA synthetase (Cyt18). This RCANA was selected from a pool of randomized sequences spanning the catalytic core of L1 ligase by selecting for the ability to ligate an oligonucleotide tag in the presence of the Cyt18 effector and affinity capture of the oligonucleotide tag. The in vitro selection can be automated by immobilization of targets on beads and high-stringency washes to remove non-binding species. Activity of another protein-dependent ribozyme was increased 3,500-fold in the presence of hen egg white lysozyme. The lysozyme-dependent ribozyme was also activated by turkey egg white lysozyme but not by T4 lysozyme and was inhibited by a lysozyme-specific RNA binding species. A peptide-dependent RCANA was isolated with an 18,000-fold increase in its activity in the presence of the arginine-rich motif (ARM) from the HIV-1 Rev protein but not the ARM from HTLV-I Rex protein.

L22 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:435215 CAPLUS
DOCUMENT NUMBER: 139:32500
TITLE: Methods for selection and use of regulatable, catalytically active nucleic acids (RCANA) or aptazymes
INVENTOR(S): Ellington, Andrew D.; Hesselberth, Jay; Marshall, Kristin A.; Robertson, Michael P.; Sooter, Letha; Davidson, Eric; Cox, J. Colin; Reidel, Timothy
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 69 pp., Cont.-in-part of U.S. Provisional Ser. No. 282,097.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003104520	A1	20030605	US 2001-883119	20010614

PRIORITY APPLN. INFO.: US 2000-212097P P 20000615

AB Compns. and methods are provided to make, isolate, characterize and use regulatable, catalytically active nucleic acids (RCANA). RCANA may be used for regulating gene expression and in assays to detect the presence of ligands or to detect activation by an effector of an RCANA bound to a solid support such as a chip or multi-well plate. One example of the invention involves construction of an RCANA by PCR using primers from the P6 region of the Group I ribozyme, cloning of the RCANA or in vitro transcription followed by RNA purifn., and demonstration of **theophylline**-dependent splicing activity towards the bacteriophage T4 gene **td intron** in vivo or in vitro. Regulatable ribozymes have been described, wherein the activity of the ribozyme is regulated by a ligand-binding moiety. Upon binding the ligand, the ribozyme activity on a target RNA is changed. Regulatable ribozymes have only been described for small mol. ligands such as org. or inorg. mols. Regulatable ribozymes that are controlled by proteins, peptides, or other macro-mols. Thus, the present invention is directed to RCANA that transduce mol. recognition into catalysis. Also disclosed are compns. and methods for automating the selection procedures of the present invention.

L22 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:924000 CAPLUS
DOCUMENT NUMBER: 136:66194
TITLE: Methods for selection and use of regulatable, catalytically active nucleic acids (RCANA) or aptazymes
INVENTOR(S): Ellington, Andrew D.; Hesselberth, Jay; Marshall, Kris; Robertson, Michael; Sooter, Letha; Davidson, Eric; Cox, J. Colin; Reidel, Timothy
PATENT ASSIGNEE(S): Board of Regents the University of Texas System, USA
SOURCE: PCT Int. Appl., 126 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096559	A2	20011220	WO 2001-US19302	20010614
WO 2001096559	A3	20030710		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,

HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-212097P P 20000615

AB Compns. and methods are provided to make, isolate, characterize and use regulatable, catalytically active nucleic acids (RCANA). RCANA may be used for regulating gene expression and in assays to detect the presence of ligands or to detect activation by an effector of an RCANA bound to a solid support such as a chip or multi-well plate. Also disclosed are compns. and methods for automating the selection procedures of the present invention. In addn., the invention claims diagnostic and therapeutic applications. One example of the invention involves construction of an RCANA by PCR using primers from the P6 region of the Group I ribozyme, cloning of the RCANA or in vitro transcription followed by RNA purifn., and demonstration of **theophylline**-dependent splicing activity towards the bacteriophage T4 gene **td intron** in vivo or in vitro. As another example of the invention, an RCANA was isolated with an activity that was increased 75,000-fold in the presence of its protein effector, Neurospora crassa mitochondrial tyrosyl tRNA synthetase (Cyt18). This RCANA was selected from a pool of randomized sequences spanning the catalytic core of L1 ligase by selecting for the ability to ligate an oligonucleotide tag in the presence of the Cyt18 effector and affinity capture of the oligonucleotide tag. The in vitro selection can be automated by immobilization of targets on beads and high-stringency washes to remove non-binding species. Activity of another protein-dependent ribozyme was increased 3,500-fold in the presence of hen egg white lysozyme. The lysozyme-dependent ribozyme was also activated by turkey egg white lysozyme but not by T4 lysozyme and was inhibited by a lysozyme-specific RNA binding species. A peptide-dependent RCANA was isolated with an 18,000-fold increase in its activity in the presence of the arginine-rich motif (ARM) from the HIV-1 Rev protein but not the ARM from HTLV-I Rex protein.

L24 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:73968 BIOSIS
DOCUMENT NUMBER: PREV200300073968
TITLE: Group I aptazymes as genetic regulatory switches.
AUTHOR(S): Thompson, Kristin M.; Syrett, Heather A.; Knudsen, Scott M.; Ellington, Andrew D. (1)
CORPORATE SOURCE: (1) Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, 78712, USA: kthompson@archemix.com, angelita@mail.utexas.edu, sknud@mail.utexas.edu, andy.ellington@mail.utexas.edu USA
SOURCE: BMC Biotechnology, (December 4 2002) Vol. 2, No. 21 Cited December 27, 2002, pp. No Pagination. http://www.biomedcentral.com/1472-6750. online. ISSN: 1472-6750.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Background: Allosteric ribozymes (aptazymes) that have extraordinary activation parameters have been generated in vitro by design and selection. For example, hammerhead and ligase ribozymes that are activated by small organic effectors and protein effectors have been selected from random sequence pools appended to extant ribozymes. Many ribozymes, especially **self-splicing introns**, are known control gene regulation or viral replication in vivo. We attempted to generate Group I **self-splicing introns** that were activated by a small organic effector, **theophylline**, and to show that such Group I aptazymes could mediate **theophylline**-dependent splicing in vivo. Results: By appending aptamers to the Group I **self-splicing intron**, we have generated a Group I aptazyme whose in vivo splicing is controlled by exogenously added small molecules. Substantial differences in gene regulation could be observed with compounds that differed by as little as a single methyl group. The effector-specificity of the Group I aptazyme could be rationally engineered for new effector molecules. Conclusion: Group I aptazymes may find applications as genetic regulatory switches for generating conditional knockouts at the level of mRNA or for developing economically viable gene therapies.

L24 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:22547 CAPLUS
DOCUMENT NUMBER: 138:282224
TITLE: Group I aptazymes as genetic regulatory switches
AUTHOR(S): Thompson, Kristin M.; Syrett, Heather A.; Knudsen, Scott M.; Ellington, Andrew D.
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, 78712, USA
SOURCE: BMC Biotechnology [online computer file] (2002), 2, No pp. given
CODEN: BBMIE6; ISSN: 1472-6750
URL: http://www.biomedcentral.com/1472-6750/2/21
PUBLISHER: BioMed Central Ltd.
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English
AB Allosteric ribozymes (aptazymes) that have extraordinary activation parameters have been generated in vitro by design and selection. For example, hammerhead and ligase ribozymes that are activated by small org. effectors and protein effectors have been selected from random sequence pools appended to extant ribozymes. Many ribozymes, esp. **self-splicing introns**, are known control gene regulation or viral replication in vivo. We attempted to generate Group I **self-splicing introns** that were activated by a small org. effector, **theophylline**, and to show that such Group I aptazymes could mediate **theophylline**-dependent splicing in vivo. By

appending aptamers to the Group I **self-splicing intron**, we have generated a Group I aptazyme whose in vivo splicing is controlled by exogenously added small mols. Substantial differences in gene regulation could be obsd. with compds. that differed by as little as a single Me group. The effector-specificity of the Group I aptazyme could be rationally engineered for new effector mols. In conclusion, group I aptazymes may find applications as genetic regulatory switches for generating conditional knockouts at the level of mRNA or for developing economically viable gene therapies.

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AB Background: Allosteric ribozymes (aptazymes) that have extraordinary activation parameters have been generated in vitro by design and selection. For example, hammerhead and ligase ribozymes that are activated by **small organic effectors** and protein effectors have been selected from random sequence pools appended to extant ribozymes. Many ribozymes, especially **self-splicing introns**, are known control gene regulation or viral replication in vivo. We attempted to generate Group I **self-splicing introns** that were activated by a **small organic effector**, theophylline, and to show that such Group I aptazymes could mediate theophylline-dependent splicing in vivo. Results: By appending aptamers to the Group I **self-splicing intron**, we have generated a Group I aptazyme whose in vivo splicing is controlled by exogenously added small molecules. Substantial differences in gene regulation could be observed with compounds that differed by as little as a single methyl group. The effector-specificity of the Group I aptazyme could be rationally engineered for new effector molecules. Conclusion: Group I aptazymes may find applications as genetic regulatory switches for generating conditional knockouts at the level of mRNA or for developing economically viable gene therapies.